

BIOLOGICAL MANAGEMENT OF GROUNDNUT STEMROT PATHOGEN

SCLEROTIUM ROLFSII (SACC.) BY *TRICHODERMA VIRIDE*

K. YAMUNARANT¹ & M. MUTHAMIZHAN²

¹Assistant Professor, Plant Pathology, Agricultural College and Research Institute, Eachangkottai,
Thanjavur, Tamil Nadu, India

²Professor, Department of Plant Pathology, Tamil Nadu Agricultural University,
Coimbatore, Tamil Nadu, India

ABSTRACT

Native isolates of groundnut stem rot casual organism, *Sclerotium rolfsii* Sacc. were collected from major groundnut growing areas of Tamil Nadu. Selected isolates were screened, characterized and identified the virulent isolate. Several native fungal antagonists were isolated against *Sclerotium rolfsii* Sacc. Among the isolates three *Trichoderma viride* and one *Trichoderma harzianum* isolate were found to have antagonistic effects on groundnut stem rot pathogen. Morphology and spore structure of isolated antagonists were studied under Light microscopy, the antagonistic activities of *Trichoderma viride* were found to be effective in reducing the mycelial growth, sclerotial formation and germination. The scanning electron microscopy revealed significant inhibition of *S. rolfsii* by complete parasitization of germinating sclerotia and malformation of sclerotia. The *Trichoderma viride* treated cultures were shown reduced mycelial growth (83.06%) and reduced sclerotial production (86.31%). The colonization behavior studies revealed that the speed of overgrowth on pathogen was high (grade 5.00) in GNTV1, GNTV2 and GNTV3 than other isolate. The higher propagule lysis was observed in all the isolates. The antimicrobial components of *Trichoderma viride* were also found to be inhibited the growth activities of the pathogen. This study provides a theoretical and practical explanation of an antagonist explored for control of stem rot caused by *S. rolfsii*.

KEYWORDS: *Sclerotium Rolfsii*, *Trichoderma Viride*, Groundnut Stem Rot & Biological Control

Received: Jan 02, 2019; **Accepted:** Jan 22, 2019; **Published:** Jan 28, 2019; **Paper Id.:** IJBRDJUN20192

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an important oilseed crop of India and it is cultivated in about 41,52,500 hectares and produce 70,77,397 MT with an average yield of 1,704 kg/ha. In spite of their important positions in the national agricultural economy and the multiplicity of crops and crop growing situations, the countries out of oilseeds are lagging far behind the requirement. The groundnut production is constrained by various factors and the major constraints include as frequent drought stress, low input use and socio-economic infrastructure and higher incidence of disease and pest attack. Though the groundnut is attacked by number of diseases, the soil borne fungal disease, stem rot caused by *Sclerotium rolfsii* is a potential threat to groundnut production and it causes yield losses over 25 %. The wide host range, profile growth and ability to produce persistent sclerotia contribute the large economic losses associated with the pathogen. Understanding the pathogen, developing and relay on single antagonism become challengeable and give way to explore and identify the suitable alternate antagonist against the disease. There are well known antifungal biocontrol agents that inhibit several plant

pathogenic fungi.

MATERIALS AND METHODS

Screening of Rhizosphere Fungal Antagonists Against the Mycelial Growth of *S. rolfsii* (Dual Culture Technique)

Six isolates of *T. viride* and four isolates of *T. harzianum* were screened against *S. rolfsii* by dual culture method (Dennis and Webster, 1971a). An actively growing 9 mm PDA culture disc of the test antagonists was cut using sterilized cork borer and placed at one end of the sterile Petri dish containing 20 ml of sterilized PDA medium previously poured and solidified under aseptic condition. A 9 mm mycelial disc of *S. rolfsii* was placed opposite end of the plate 1.5 cm away from the edge of the plate and incubated at room temperature ($28 \pm 2^\circ\text{C}$). The Petri dishes were maintained for each antagonist separately. The medium inoculated with the pathogen alone was served as control. When the control plate reached full growth, the radial growth of the pathogen and inhibition zone was measured in the other treatments. The results were expressed as per cent inhibition over control by using the formula of Pandey *et al.* (2000). The overgrowth of antagonists over the pathogen was measured seven days after incubation. The overgrowth and zone of inhibition were measured and expressed in cm.

$$\text{PI} = \frac{\text{Dc} - \text{Dt}}{\text{Dc}} \times 100$$

Dc = average diameter of fungal growth (cm) in control

Dt = average diameter of fungal growth (cm) in treatment.

Growth Characters of the *T. viride* Isolates

The best isolates of *T. viride* (GNTV1, GNTV2, GNTV3 and GNTV4,) screened by dual culture as in above were taken for further studies. A 9 mm mycelial disc of each isolate was inoculated at the centre of the PDA medium and incubated at room temperature. The linear growth was measured after 48 h and 72 h of incubation. For bio-mass production a 9 mm mycelial disc of each isolate of *T. viride* was inoculated to 100 ml conical flask containing 25 ml of sterilized PDB. After seven days of incubation, the mycelial mat was filtered through a Whatman No.41 filter paper and the dry weight was estimated. The other characters such as colony colour, days taken for sporulation were also observed.

Colonization Behaviour (CB) (Bell *et al.*, 1982)

A 9 mm mycelial disc of *Sclerotiumrolfsii* and *T. viride* isolates of, GNTV2, GNTV3 and GNTV4 were placed opposite to each other near the periphery of the Petridish. The plates were incubated at room temperature. The colonization behaviour of the isolate was assessed by using the grades 1 – 5.

Grade 1 = Pathogen partially/ of completely overgrow on *T. viride*.

Grade 2 = Locked at the point of contact of pathogen and antagonist.

Grade 3 = Initiation of overgrowth of antagonist on pathogen.

Grade 4 = 75 per cent over growth of antagonist on pathogen.

Grade 5 = Complete overgrowth of antagonist on pathogen.

Propagulelysis (PL) (Kasinathan, 1986)

From the zone of interaction of the *T.viride* antagonists (GNTV1, GNTV2, GNTV3 and GNTV4) and the pathogen five numbers of five mm discs were excised (*Sclerotiumrolfsii*) and plated on PDA. Based on the survival nature and lysis of *S.rolfsii* a grading system of 1 to 5 was used to express the propagulelysis.

Grade 1 = Hyphae of > 50 % on the agar surface surrounding the disc.

Grade 2 = Hyphae of 26-50 % on the agar surface surrounding the disc.

Grade 3 = Hyphae of 1-25 % on the agar surface surrounding the disc.

Grade 4 = several strands of hyphae.

Grade 5 = No observable hyphae.

Speed of Overgrowth on the Pathogen (SOOP) (Bell *et al.*, 1982)

Time taken by the antagonist or the pathogen after contact both in dual culture plates was assessed as speed of over growth using the class 1- 4.

1 = *S. rolfsii* overgrow on *Trichoderma*.

2 = Neither *Trichoderma* nor pathogen overgrow on each other.

3 = *Trichoderma* completely overgrow on *S.rolfsii* after 48 h.

4 = *Trichoderma* completely overgrow on *S.rolfsii* within 24- 48 h.

Inhibition Zone (IZ) (Bell *et al.*, 1982)

Inhibition zone was measured in dual culture plates of *Sclerotiumrolfsii* and *T.viride* and it was categorized into four classes.

Class 1= No inhibition zone,

Class 2 = 1 to 2.5 mm,

Class 3 = 2.6 to 5 mm,

Class 4 = > 5 mm

Effect on Germination of Sclerotia

Isolates of *T.viride* (GNTV1, GNTV2, GNTV3 and GNTV4) were tested for their effect on germination of sclerotia in natural soil plates. Fifty gram of field soil, adjusted to 80% moisture content was evenly distributed in Petri dishes and slightly compacted. Before placement on the soil, sclerotia were immersed for 24 hours in culture filtrates of the isolates of *T.viride* separately. Sclerotia (20 numbers per plate) were equally placed on the soil surface and pushed into the soil gently with glass rod so that only their tops remain exposed. Plates were incubated at room temperature for seven days and examined for its germination. (Henis *et al.*, 1984).

Effect of Volatile Compounds

Effect of volatile metabolites produced by *T.viride* (GNTV1, GNTV2, GNTV3 and GNTV4,) on mycelial growth of *S. rolfii* was studied by Paired Petri dish technique (Laha *et al.*, 1996). A 9 mm mycelial disc of four day old culture of *T.viride* isolates was placed on PDA medium in a Petri dish. The top of each Petri plate was replaced with bottom of PDA plates inoculated centrally with 9 mm diameter mycelial disc of *S.rolfsii* and inverted over the antagonists *T.viride* plates and sealed with Para film and incubated at room temperature for four days. PDA plates inoculated with the pathogen alone and paired with PDA plate without bio-control agents served as control. Three replications were maintained in each treatment. The mycelial diameter of the pathogen was measured after incubation of four days and expressed as per cent inhibition over control.

Extraction of Crude Antibiotic from Antagonistic Fungi

Cultures of *T. viride* were grown at $28\pm 2^{\circ}\text{C}$ in Pigment Production Medium (PP) (Peptone-20g/l, Glycerol-20g/l, NaCl-5g/l, KNO_3 -1g/l, pH-7.2, Distilled Water-1litre). The cultures were grown in Pigment production broth for five days and were centrifuged at 5000 rpm and the supernatants were adjusted to pH 2.0 with conc. HCl and it was extracted with an equal volume of benzene. The Benzene layer was subjected to evaporation in water bath. After evaporation, the residues were resuspended in 0.1N NaOH.

Extraction of Antibiotic with Ethyl Acetate *T. viride*

Cultures of *T. viride* were grown in 100ml of Pigment Production broth for four days on a rotary shaker at 30°C . The fermented broth was centrifuged at 3500 RPM for five minutes in a tabletop centrifuge and the supernatant was collected. It was acidified to pH 2.0 with 1N HCl and then extracted with an equal volume of ethyl acetate. The ethyl acetate extracts were reduced to dryness *in vacuo*. The residues were dissolved in methanol.

Effect of Benzene Fraction and Ethyl Acetate Fraction on the Growth of *S. Rolfii*

The crude residues of Benzene fraction and ethyl acetate fraction were evaluated at 0.1% and 0.5% concentration for their inhibitory effect on the mycelial growth of *S. rolfii* by poisoned food technique (Schmitz, 1930).

RESULTS AND DISCUSSION

Characterization of *T.viride* Isolates

The fungal antagonists having high rhizosphere competence was screened by taking rhizosphere soils from different locations against the groundnut stem rot *S. rolfii*. The four native isolates of *T.viride* (Ground nut *Trichoderma viride*) viz., GNTV1, GNTV2, GNTV3 and GNTV 4 were found to be effective against *S. rolfii* (Table 1). This is in accordance with the study of Anand Singh and HarikeshBahadur (2004) who reported eight isolates of different species of *Trichoderma* and two isolates of *Gliocladium virens* were effective isolates for stem rot management.

Characterization and Colonization behavior were studied to find out effective fungal antagonist against *S.rolfsii*. The isolate GNTV1 recorded highly ramified mycelium with dark green colonies and sporulated earlier 2.5 days (than other isolates 3-4 days) with higher mycelial growth of 90 mm within 48 hours which are preferred for a competent antagonist (Table 2). In respect of *S. rolfii* inhibition, the earlier study by Weindling (1932) demonstrated the similar results with fungal antagonist *Trichoderma* spp. Therefore the native fungal antagonist GNTV1 was taken as promising agent against *S. rolfii*. Sarita *et al.*, 2018 reported

T. harzianum is very effective in reducing the radial growth of *S. rolfsii*. The further studies on antagonistic activities of GNTV1 revealed that, it had positive antagonistic characters like highest mycelial growth inhibition of 83.06 per cent over control and minimum production of sclerotia (28.04 numbers/ plate). Dwivedi and Ganesh Prasad (2018) reported *S. rolfsii* may be controlled through biological agents (*Trichoderma harzianum*, *T. viride*, *T. asperellum*)

The culture filtrate of Tv1 inhibited the growth of the pathogen (87.05%) as well as sclerotial germination (62.50%) to a greater extent than others. Uma Maheswari et al., 2002 observed the similar results of *T. viride* control measures. Srinivasulu et al., (2005) observed the mycelial interaction between *Trichoderma* spp. and *S. rolfsii* and found that highest inhibition of mycelial growth (52.22 per cent) was recorded in case of *T. hamatum* followed by *T. viride* (44.11 per cent).

Effect of volatiles and antibiotics of fungal antagonist against *S. rolfsii* was studied. GNTV1 was compared with other isolates and found to be reducing mycelial growth of *S. rolfsii* by 53.41 per cent (Table 3) (Table 4). The fungicidal effect of volatile metabolites of *T. viride* was already proved. Srinivasulu et al., (2005) reported that, all the three *Trichoderma* spp. i.e., *T. viride*, *T. hamatum* and *T. harzianum* are very effective in producing volatile substances against *S. rolfsii*. Among crude antibiotic and ethyl acetate fraction eluted and tested, the crude antibiotic isolated from GNTV1 recorded higher inhibition of 13.67 per cent which was 3.67 per cent more than the ethyl acetate fraction.

CONCLUSIONS

Several native fungal antagonists were isolated against *Sclerotium rolfsii* Sacc. Among the isolates three *Trichoderma viride* and one *Trichoderma harzianum* isolate was found to have antagonistic effects on groundnut stemrot pathogen. Morphology and spore structure of isolated antagonists were studied under Light microscopy, the antagonistic activities of *Trichoderma viride* were found to be effective in reducing the mycelial growth, sclerotial formation and germination. The scanning electron microscopy revealed significant inhibition of *S. rolfsii* by complete parasitization of germinating sclerotia and malformation of sclerotia. The *Trichoderma viride* treated cultures were shown reduced mycelial growth (83.06%) and reduced sclerotial production (86.31%). The colonization behavior studies revealed that the speed of overgrowth on pathogen was high (grade 5.00) in GNTV1, GNTV2 and GNTV3 than other isolate. The higher propagule lysis was observed in all the isolates. The antimicrobial components of *Trichoderma viride* were also found to be inhibited the growth activities of the pathogen.

REFERENCES

1. Anand Singh and Harikesh Bahadursingh. 2005. Control of collar rot in mint (*Mentha* spp.) caused by *Sclerotium rolfsii* using biological means. *Cur. Sci.*, **87**(3): 362-366.
2. Bell, D.K., H.D. Wells and C.R. Markham. 1982. In vitro antagonism of *Trichoderma* spp. against six fungal plant pathogens. *Phytopathology*, **72**: 379-382.
3. Dennis, C. and J. Webster. 1971. Antagonistic properties of species of *Trichoderma* I. Production of non-volatile antibiotics. *Trans. Br. Mycol. Soc.*, **57**: 25-39.
4. Dwivedi, S. K. and Ganesh Prasad. 2016. Integrated Management Of *Sclerotium Rolfsii*. *European Journal of Biomedical and Pharmaceutical Sci.*, **3**(11): 137-146
5. Henis, Y., J.A. Lewis and G.C. Papavizas. 1982. Penetration of sclerotia of *Sclerotium rolfsii* by *Trichoderma* spp. *Phytopathol.*, **73**: 1043-1046.

6. Laha, G.S., J.P. Verma and R.P. Singh. 1996. Effectiveness of fluorescent pseudomonads in the management of sclerotial wilt of cotton. *Indian Phytopathol.*, **49** (1): 3-8.
7. Pandey, K.K and J.P. Upadhyay. 2000. Microbial population from rhizosphere and non –rhizosphere soils of pigeonpea screening for resident antagonist and mode of mycoparasitism. *J. Mycol. Pl. Pathol.*, **30**(1): 7-10.
8. Sarita, Shankar Soyal and R.S.Ratnoo. 2018. Bio-efficacy and management of stem rot (*Sclerotium rolfsii*) of groundnut with different fungicides and bioagents. *Indian Journal ChemSci.*, **6**(5): 492-495
9. Schmitz, H. 1930. *Poisoned food technique Industrial and Engineering Chemistry*. Analyst, 2: 361.
10. Srinivasulu, B., K. V. Krishna kumar., K. Aruna., J. Krishna prasadji and D. V. R. Rao. 2005. In vitro antagonism of three *Trichoderma* spp. Against *Sclerotium rolfsii* Sacc., a collar- rot pathogen in elephant foot yam. *J. Biol. Control*, **19** (2): 161-171.
11. Weindling, R. 1934. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. *Phytopathol.*, **24**: 1153-1179.

APPENDICES

Table 1: Characters of *T. viride* Isolates on Solid and Liquid Media

S.No	Isolate	Solid Media			Liquid Media			
		Colony Colour	*Mycelial Diameter (mm)		No. of Days Taken for Sporulation	No. of Days Taken for Full Growth	No. of Days Taken for Sporulation	Mycelial Dry Weight (mg)
			48 h	72h				
1	<i>T. viride</i> (GNTV1)	dark green	90.00	90.00	2.5	4.0	6.0	310.00
2	<i>T. viride</i> (GNTV2)	Light green	90.00	90.00	3.0	5.0	7.0	300.00
3	<i>T. viride</i> (GNTV3)	light green	75.00	85.00	4.0	5.0	7.0	260.00
4	<i>T.harzianum</i> (GNTH1))	light green	75.00	90.00	4.0	6.0	8.0	280.00
CD (P=0.05)		Treatments	0.87					
		Growth	0.61					
		T X G	1.23					

* Mean of three replications

Table 2: Antagonistic Property of *T. viride* Against *S. rolfsii* in vitro

S. No	Isolate	Colonization Behaviour(Grade)	Propagule Lysis (Grade)	Speed of Over Growth on Pathogen (Grade)	No. of Sclerotia / Plate*	Per cent Reduction Over Control
1	<i>T. viride</i> (GNTV1)	5.00	5.00	5.00	22.33	81.66
2	<i>T. viride</i> (GNTV2)	5.00	5.00	5.00	35.33	70.96
3	<i>T. viride</i> (GNTV3)	3.00	5.00	2.00	51.33	57.80
4	<i>T. harzianum</i> (GNTH1)	5.00	5.00	5.00	60.33	50.41
5	Control	--	---	---	121.66	--
CD (P=0.05)					1.51	-

*Mean of three replications

Table 3: Effect of Volatile and Non Volatile Compounds of *T. viride* on the Growth of *S. rolfsii*

S. No	Isolates	Mycelial Diameter (cm)*	Per cent Reduction Over Control
1	<i>T. viride</i> (GNTV1)	4.16	53.41
2	<i>T. viride</i> (GNTV2)	4.33	51.51
3	<i>T. viride</i> (GNTV3)	6.67	25.30
4	<i>T. harzianum</i> (GNTH1)	6.83	23.51
5	control	8.93	-
CD (P=0.05)		2.06	-

* Mean of three replications

Table 4: Effect of Crude Antibiotics of *Trichoderma viride* on *S. rolfsii*

S. No.	Antibiotics	Mycelial Diameter (mm)		Per cent Reduction Over Control	
		Concentration 0.1%	Concentration 0.5%	Concentration 0.1%	Concentration 0.5%
1	Benzeneextract <i>Trichoderma viride</i> (GNTV1)	87.60	78.6	2.70	12.67
2	<i>Trichoderma harzianum</i> (GNTH1)	89.30	81.60	0.70	9.30
3	Ethyl acetate fraction <i>Trichoderma viride</i> (GNTV1)	88.26	77.70	1.93	13.67
4	<i>Trichoderma harzianum</i> (GNTH1)	88.00	81.00	2.22	10.00
5	Control	90.00	90.00	--	--

CD (P= 0.05)

Antibiotics = 2.79
 Concentration =1.76
 A x C = 3.95

